Docket No.: 01017/40451B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Brockhaus et al.

Application No.: 08/444,790 Art Unit: 1646

Filed: May 19, 1995 Examiner: Zachary Howard

For: HUMAN TNF RECEPTOR

DECLARATION OF TARUNA ARORA, PH.D. UNDER 37 C.F.R 1.132

1. For the last seven years, I have been employed as a scientist at Amgen Inc., and currently am a Principal Scientist. I hold a Ph.D. in Immunology from the Mayo Clinic in Rochester, Minnesota, and performed post-doctoral research at UCLA in the Department of Hematology.

2. I make this declaration to describe the results of experiments that I directed. In these experiments, we assayed the activity of several proteins in a complement mediated cytotoxity (CDC) assay and an antibody dependent cellular cytotoxicity (ADCC) assay. These proteins were etanercept (the extracellular domain of the p75 TNF receptor fused to all of the hinge, CH2, and CH3 domains of an IgG1 heavy chain constant region), infliximab (a chimeric anti-TNF antibody), adalimumab (a human anti-TNF antibody), and two different alternative fusion proteins named 3.5D and Delta 57.

3. The amino acid sequence of fusion protein 3.5D is shown on Exhibit A. This fusion protein has amino acids 1 to 163 of the mature p75 TNF receptor fused via a 27 amino acid glycine-rich linker (in bold type) to a portion of an IgG1 hinge

domain, and all of an IgG1 CH2 and CH3 domain. This construct is missing the first 5 amino acids of the hinge domain (EPKSC).

4. The amino acid sequence of fusion protein Delta 57 is shown in Exhibit B. This fusion protein has amino acids 1 to 179 of the mature p75 TNF receptor fused via a 27 amino acid glycine-rich linker (in bold type) to a portion of an IgG1 hinge domain, and all of an IgG1 CH2 and CH3 domain. Thus this construct is also missing the first 5 amino acids of the hinge domain (EPKSC).

5. Exhibit C shows the results of a CDC assay. In this assay, MT-3 cells, which are CHO cells engineered to stably express TNFα on their surface, are combined with varying amounts of the TNF antagonist test protein (as indicated) for 30 minutes at 4°C. The cells and test protein were then incubated with human complement-rich serum (Quidel) to a final concentration of 14%, for 2 hours at 37°C. The degree of cell death was determined by analysis of propidium iodide uptake by flow cytometry, and the value obtained from untreated sample was subtracted from the experimental total shown. Of the five proteins tested, only etanercept consistently showed lower CDC activity in the presence of active complement-rich serum.

6. Exhibit D shows the results of two ADCC assays using purified donor peripheral mononuclear cells (PBMCs) from two different donors as effector cells. In this assay, the target cells were MT-3 cells that have been detached from tissue culture plates and labeled with membrane-integrating pKH67dye. PBMC's were mixed with MT-3 cells at 25:1 (effector : target) ratio and incubated with varying concentrations of the TNF antagonists for 4 hours at 37°C, after which 7AAD was

added to the cells to measure cell death. The percent ADCC was calculated using the following equation: 1 – (% $pKH67^+$ cells in test molecule treated sample/ % $pKH67^+$ cells in untreated sample) x 100. The results from these two experiments varied somewhat. The variation may be due to the fact that the PBMCs used as effector cells in these two experiments came from different donors. However, the results from samples containing etanercept were consistent in that the amount of ADCC detected was comparable to that induced by a random control antibody (anti DNP hIgG1) that does not bind TNF. Thus, these results indicate that etanercept mediates little or no ADCC.

7. I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. § 1001), and may jeopardize the validity of the application or any patent issuing thereon.

Dated: Dec 16, 2010 Tamra Arora

EXHIBIT A

Protein 3.5D

lpaqvaftpyapepgstcrlreyydqtaqmccskcspgqhakvfctktsdtvcdscedstytqlwnwvpeclscgsrcssdqvet
qactreqnrictcrpgwycalskqegcrlcaplrkcrpgfgvarpgtetsdvvckpcapgtfsnttsstdicrphqic**ggnnsggsg**
gsggnnsgggsgggnnsdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvev
hnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlppsreemtknqvsltclv
kgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

EXHIBIT B

Protein Delta 57

lpaqvaftpyapepgstcrlreyydqtaqmccskcspgqhakvfctktsdtvcdscedstytqlwnwvpeclscgsrcssdqvet
qactreqnrictcrpgwycalskqegcrlcaplrkcrpgfgvarpgtetsdvvckpcapgtfsnttsstdicrphqicnvvaipgnas
mdavct**ggnnsggsggsggnnsgggsgggnns**dkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshe
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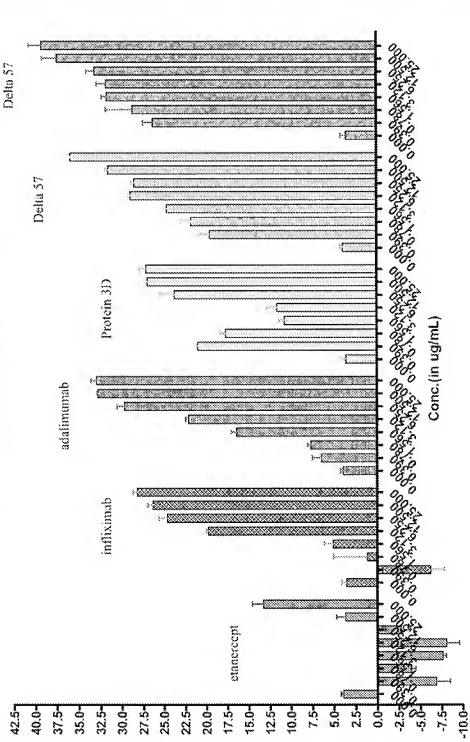
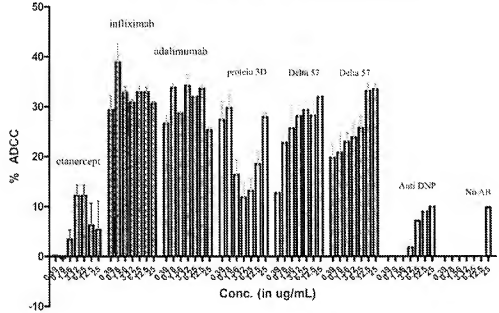


Exhibit C
CDC with Human Complement Serum
% Cell Death
Conc. (in ug/mL)
etanercept
infliximab
adalimumab
Protein 31)
Delta 57
Delta 57

Exhibit D

ADCC assay with TNF antagonists- Donor 1



ADCC assay with TNF antagonists- Donor 2